A COMPLETE DESCRIPTION OF CLOSTRIDIUM PUTREFACIENS (McBRYDE)

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More than fifteen years ago McBryde (1911) isolated an interesting anaerobe differing in several characters from any previously described. He suggested the name *B. putrefaciens* for this new species. Although a considerable description of this organism, based presumably on a single culture, was given in this bulletin, it seems not to have received any recognition in the literature of the subsequent fifteen years. Recently Boyer (1926) reports the frequent finding of this organism, and also of other anaerobes, in animal muscle tissue soon after slaughter.

In this Laboratory during the past few years, 27 strains have been isolated from animal sources which conform with the anaerobe already described. They are strict anaerobes of the clostridial type and are markedly proteolytic. Young cultures in meat media are characterized by filaments and chains. After two or three days cultivation the cells become club-shaped, this deep-staining terminal swelling being best described as bulbed. From this initial stage sporulation proceeds rapidly and mature spores are found a day or two later. They are plectridial, being terminal, spherical, and about 2.2 times the diameter of the rod. The typical drumstick sporangia remain intact for several weeks, during which time free spores are extremely rare. When found these are faintly stained (Gram method) and are apparently thin-walled. Very old cultures show considerable disintegration of cells with the liberation of many of these faint-staining free spores.

While this is the typical behavior of *C. putrefaciens* when first

cultured in the laboratory, it must be admitted that the writers in common with Boyer (1926) have noted a considerable lessening of sporulation after prolonged cultivation of pure cultures. No other changes in the behavior have been observed.

A detailed study of these 27 strains based on the latest S. A. B. Descriptive Chart has been made and is now offered in confirmation of the original work of McBryde. The various refinements of methods which have developed within the past fifteen years have

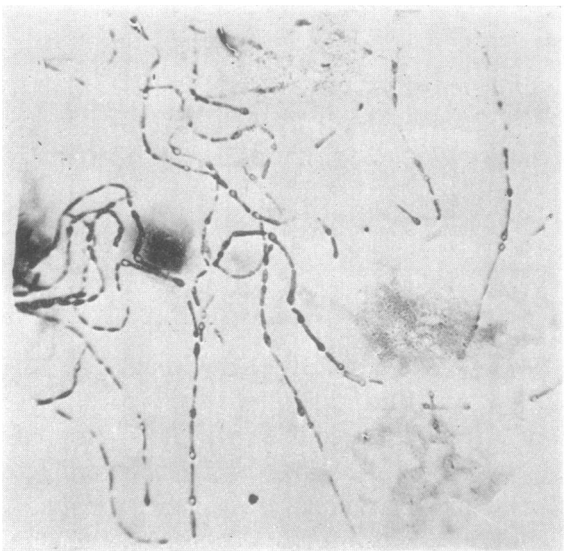

FIG. 1. CLOSTRIDIUM PUTREFACIENS. OLD CULTURE IN PORK MEDIUM WITH AN INITIAL pH OF 8.0. × 1200

been employed in our attempt to establish this unique organism among the anaerobic clostridia where it undoubtedly belongs.

Morphology. Rods 0.5–0.7 by 3–15μ with rounded ends, occurring singly and in chains. Drumstick sporangia with spherical terminal spores 1.1–1.6μ in diameter. Non motile. Gram positive. Thick filaments predominate in low pH media.

Agar colonies. Small, filamentous.

Agar slant. Scanty, white, beaded, glistening.

Broth. Moderate, transient, turbidity; heavy growth occurring as a flocculent sediment at bottom.

Minced pork. Slight disintegration of meat particles with strong, characteristic, sour, putrefactive odor. There is little visual evidence of digestion of the meat until the tube is shaken vigorously when the contents disintegrate into a more or less homogeneous pulp.

Gelatin stab. Growth and liquefaction starting at bottom in two to three days. Liquefaction complete in three to five days.

Litmus milk. Litmus reduced in four to seven days. Rennet curd appears in fifteen days at a pH of 6.5. Peptonization follows slowly.

Indol not formed.

Hydrogen sulphide. Slight production.

Nitrates not reduced.

Fermentation. Acid and slight gas in glucose. No acid or gas in lactose, sucrose or maltose. Starch not hydrolyzed.

Anaerobic. No surface growth on any medium without strict anaerobiosis.

Temperature relations. Optimum 20° to 25°C. Slow growth at 0°C. and even at lower temperatures. No visible growth at 37°C.

pH relations. Growth occurs in meat medium between pH 6.0 and pH 9.0 with an optimum at pH 8.0.

Habitat. Found in muscle tissues of hogs at slaughter.

In common with McBryde we have found pork extract to be favorable for growth, and it has been used as the basis of the agar, gelatin, and broth media employed. Anaerobic conditions were obtained by twice evacuating to the vapor pressure of water and refilling with hydrogen.

It will be noted that our negative findings for indol are at variance with McBryde's. Cultures in both pork extract pepton broth and tryptophane broth (Bacto) have been tested at the ages of one, three and seven days by the Ehrlich test. A slight positive test was obtained when the cultures were tested directly. It was found, however, that the color could not be extracted with chloroform. Duplicate cultures were subjected to a steam distillation according to the technique of Fellers and Clough (1925). No positive test could be obtained by this method.

From the foregoing study it is evident that *C. putrefaciens* is a most interesting anaerobe,—so far as the writers are aware

unique in its temperature requirements. This strongly proteolytic organism grows well at 0°C. and little or not at all at 37°C.

Morphologically the typical drumstick sporangia are suggestive of *C. putrificum* (Bienstock) and *C. tetani*. The rods of *C. putrefaciens*, however, have a slightly greater diameter than those of *C. putrificum* and often exceed those of *C. tetani* in length. Furthermore the lack of motility and the low temperature requirements of the first species differentiate it from the two latter.

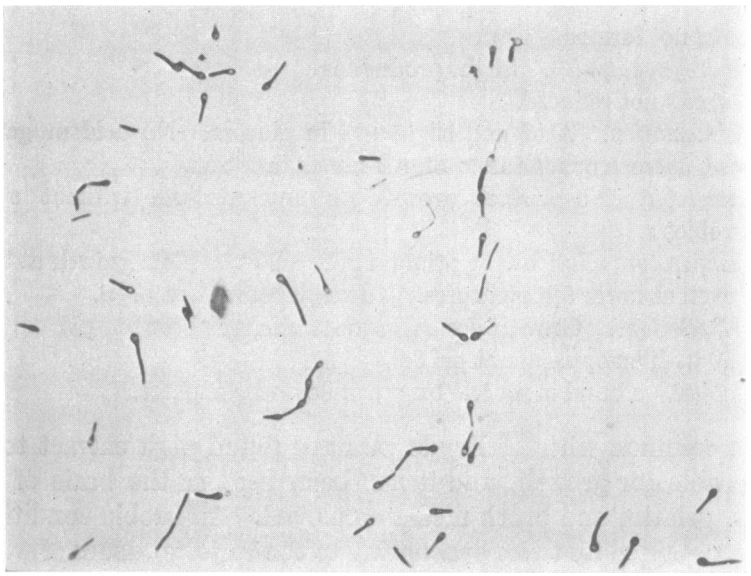

FIG. 2. CLOSTRIDIUM PUTREFACIENS. GROWN TEN DAYS IN PORK MEDIUM WITH AN INITIAL pH OF 6.8. × 1200.

The odor produced in meat is very characteristic. One accustomed to these odors has no difficulty in differentiating pure cultures of this anaerobe from other putrefactive organisms by this means.

Old meat cultures of *C. putrefaciens* present a sharp contrast with similar cultures of other putrefactive anaerobes in which extensive liquefaction is perfectly evident. A marked softening of the meat without evident reduction in bulk is characteristic of *C. putrefaciens*.

The twenty-seven strains studied, although isolated from different sources, show perfect agreement on all characteristics. This agreement in cultural and physiological details, the consistently characteristic and distinctive microscopic picture, and our repeated failure to obtain growth from any of the strains at 37° under anaerobic conditions, or at any temperature under aerobic conditions, constitute a part of our criteria for considering these cultures to be pure.

The writers consider this unique organism deserving of recognition as a distinct species and suggest its inclusion in the genus Clostridium, as *C. putrefaciens*.

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